iGEM 2016: Pittsburgh
Week 6 Lab Notebook

Monday, June 27

Lab meeting

Digest T7-RBS -- amilCP with PstI to linearize

Mini-prep liquid cultures of terminator ligations (Maddie)

Concentrations (ng/uL)

	1	2	3	4	5
T7eGFP-term	151.3	108.5	182.1	198.9	91.5
T7amilCP-term	161.6	143.2	112.4	149.1	137.9

Digest with EcoRI and PstI (10 uL reaction volume) (Claire)

0.5 uL EcoRI

0.5 uL PstI

1 ug DNA =

	1	2	3	4	5
T7eGFP-term	6.61 uL	9.22	5.49	5.03	10.93
T7amilCP-term	6.19	6.98	8.90	6.71	7.25

1 uL buffer

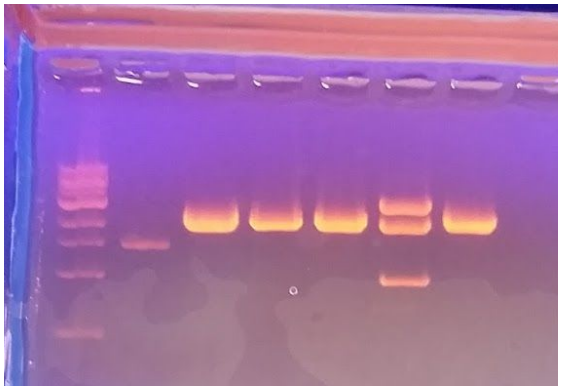
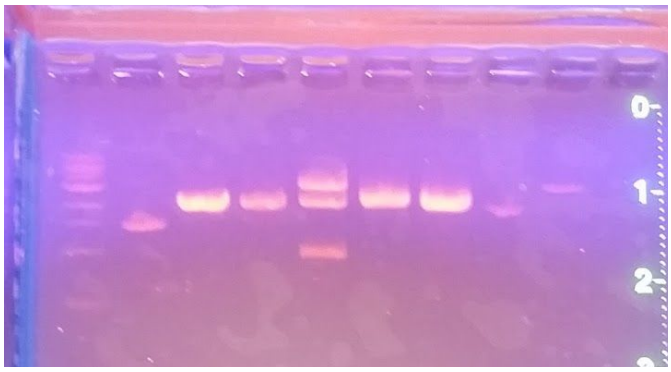
H2O to volume =

	1	2	3	4	5
T7eGFP-term	1.39 uL	0	2.51	2.97	0
T7amilCP-term	1.81	1.02	0	1.29	0.75

Gel check of ligations

Lane		
	1	ladder
	2	uncut T7eGFP-term 1
	3	1
	4	2
	5	3
	6	4
	7	5

Lane		
	1	ladder
	2	uncut T7amil-term 1
	3	1
	4	2
	5	3
	6	4
	7	5
	8	uncut T7--amil
	9	lin T7--amil 3-2

amilCP 3 and eGFP 4 are successful

Transform 5 uL successful ligation plasmid in 75 uL Cheryl's cells sitting out from Aife

Purify 3-2 T7-RBS amilCP

15 ng/uL, about 25 uL

Cell-free test of amilCP -- no color

Tuesday, June 28

No T7-eGFP-term 4 growth, redo transformation (Maddie and Claire)

Resuspend IDT oligos at 100 uM (Maddie and Claire)

VR: 289 uL water

VF2: 318 uL water

#1 DNA Trigger_Collins Swit: 678 uL

#2 DNA Trigger_Collins Swit: 580 uL

T3-RBS-suffix antisense: 281 uL

prefix-T3-RBS: 724 uL

Biobrick reverse: 313 uL

Biobrick forward: 294 uL

Sequence promising T7 amilCP term and T7 eGFP term (Claire)

2.5 uL primer

500 ng plasmid		amilCP: 4.45 uL	eGFP: 2.51 uL
H2O to 15 uL		amilCP: 8.05	eGFP: 9.99 uL
amilCP	VF2	LA01	
	VR	LA02	
eGFP	VF2	LA03	
	VR	LA04	

Cell-free test of amilCP (again) --nothing

Wednesday, June 29

Cell-free Collins toeholds experiment with 25 ng switch and decreasing trigger (Claire)

Amt D trigger	25 (x2)	25 mismatch (x2)	10 (x2)	5 (x2)	0
Solution A	2	2	2	2	2

Solution B	1.5	1.5	1.5	1.5	1.5
RNAse	0.25	0.25	0.25	0.25	0.25
Switch (13-2) 25 ng	0.30	0.30	0.30	0.30	0.30
Trigger (14-1)	0.61 at /4 dilution	0.67 **16-2** at /4 dilution	0.61 at /10 dilution	0.61 at /20 dilution	0
substrate	0.25	0.25	0.25	0.25	0.25
water	0.09	0.09	0.09	0.09	0.7

/4 dilution (40.725 ng/uL): 1 uL 14-1 + 3 uL water
/10 dilution (16.29 ng/uL): 0.5 uL 14-1 + 4.5 uL water
/20 dilution (8.145 ng/uL): 1 uL /10 + 1 uL water

Amt G trigger	25 (x2)	25 mismatch (x2)	10 (x2)	5 (x2)	0
Solution A	2	2	2	2	2
Solution B	1.5	1.5	1.5	1.5	1.5
RNAse	0.25	0.25	0.25	0.25	0.25
Switch (13-2) 25 ng	0.26	0.26	0.26	0.26	0.26
Trigger (14-1)	0.67 at /4 dilution	0.61 **14-1** at /4 dilution	0.67 at /10 dilution	0.67 at /20 dilution	0
substrate	0.25	0.25	0.25	0.25	0.25
water	0.07	0.07	0.07	0.07	0.74

/4 dilution (37.15 ng/uL): 1 uL 14-1 + 3 uL water
/10 dilution (14.86 ng/uL): 0.5 uL 14-1 + 4.5 uL water
/20 dilution (7.43 ng/uL): 1 uL /10 + 1 uL water

PROCEDURE

1. Dilute trigger plasmids to working concentrations as written under tables
2. Make master mix for 20 reactions (18 reactions + 2 for pipetting error)
    - 40 uL Solution A
    - 30 uL Solution B
    - 5 uL RNAse inhibitor
    - 5 uL Substrate (Chlorophenol Red-β-D-galactopyranoside at 12 mg/mL = 20X)

3. Separate 40 uL of master mix, 40 uL (10 rxns) each for D and G
4. Add 3 uL 13-2 switch // 2.6 uL 15-1 switch
5. Remove 4.3 uL // 4.26 uL for controls
6. To Step 5, add 0.7 uL // 0.74 uL water

CONTROLS DONE

7. From Step 4, remove 8.6 uL // 8.52 uL for mismatch reactions
8. To Step 7, add 0.06 uL // 0.26 uL water
9. Separate Step 8 into 2 rxns of 4.33 uL // 4.39 uL
10. Add 0.67 uL /4 16-2 trigger // 0.61 uL /4 14-1 trigger to each of 2

MISMATCHES DONE

11. To Step 4, add 0.63 uL // 0.49 uL water
12. Separate into 6 rxns of 4.39 uL // 4.33 uL
13. Add 0.61 uL // 0.67 uL DNA

ALL REACTIONS DONE

14. Spin down in microcentrifuge
15. Incubate at 37 degC for 2 hours

*13 5-2 ran low on mix
Color change visible by 50 minutes into incubation, not as much for 13 5-1 and 5-2
Dilute to 55 uL total

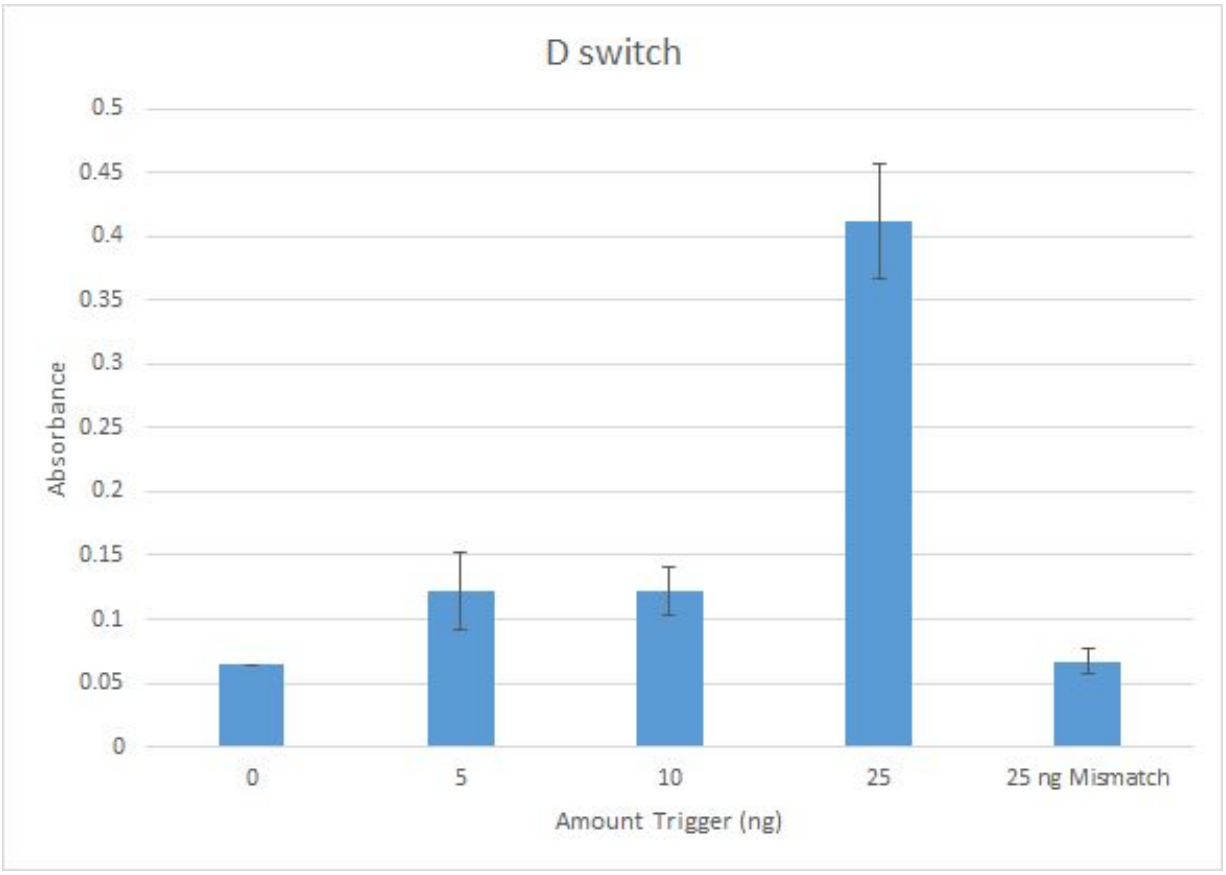
RESULTS
Clear plate:

	1	2	3	4	5	6	7	8	9
C	13-0	13 5-1	13 5-2	13 10-1	13 10-2	13 25-1	13 25-2	13 MM 1	13 MM 2
D	15-0	15 5-1	15 5-2	15 10-1	15 10-2	15 25-1	15 25-2	15 MM 1	15 MM 2

Absorbance read at 570 nm (Roy)

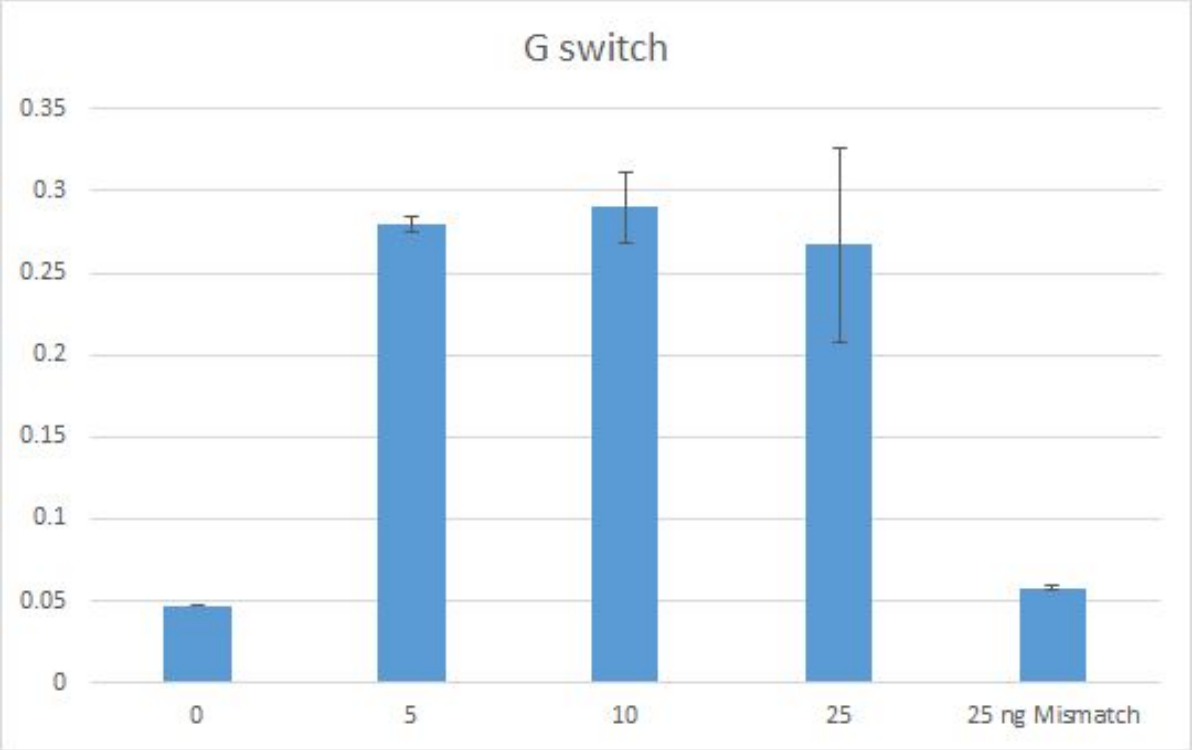
	1	2	3	4	5	6	7	8	9
C	0.064	0.143	0.100	0.136	0.109	0.443	0.380	0.074	0.060
D	0.047	0.283	0.276	0.275	0.305	0.225	0.309	0.057	0.059

D switch. From left to right: control, mismatches (x2), 5 ng (x2), 10 ng (x2), 25 ng (x2)



Absorbances read at 570 nm. 5 uL duplicate reactions were diluted to 55 uL with water to cover the bottoms of the wells. Error bars represent standard deviation.

G switch. From left to right: control, mismatches (x2), 5 ng (x2), 10 ng (x2), 25 ng (x2)



Absorbances read at 570 nm. 5 uL duplicate reactions were diluted to 55 uL with water to cover the bottoms of the wells. Error bars represent standard deviation.

Glycerol stock of T7RBS-amilCP-terminator (Maddie)
Miniprep liquid cultures (Maddie)
Concentrations (ng/uL):

T7 RBS -- amilCP	1	118.9
	2	249.6
	3	341.1
	4	191.3

T7 RBS -- amilCP -- terminator: 149.2
Make liquid cultures of T7RBS -- amilCP -- terminator (x3) (Claire)
T7RBS -- eGFP -- terminator (x4)
eGFP plate was overgrown; difficult to pick individual colonies
Test amilCP with and without terminator in cell free reaction (Aife)
Linearized: 15 ng/uL
amilCP--term 2: 243.2 ng/uL in duplicate

	200 ng	100 ng	50 ng	25 ng	25 ng linear	Control
Solution A	2	2	2	2	2	2
Solution B	1.5	1.5	1.5	1.5	1.5	1.5
RNAse inhibitor	0.025	0.025	0.025	0.025	0.025	0.025

DNA	1.4	0.7	0.35	0.17	1.6	0
water	0.75	0.775	1.125	1.305	0	1.475

No color visible to the naked eye

Thursday, June 30

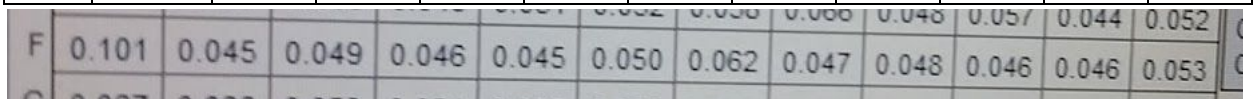
Miniprep (Maddie)

concentrations ng/uL

T7-eGFP-term1	202.6
T7-eGFP-term2	81.2
T7-eGFP-term3	99.1
T7-eGFP-term4	230.6
T7-amilCP-term1	62.3
T7-amilCP-term2	52.3
T7-amilCP-term3	51.3

Read yesterday's amilCP reactions (absorbance at 588 nm)

	1	2	3	4	5	6	7	8	9	10	11
F	control	25-1	25-2	50-1	50-2	100-1	100-2	200-1	200-2	200 lin-1	200 lin-2



No protein detected

Cell-free T7-GFP volume optimization and quick test of plasmid eGFP (Claire)

Volume optimization with T7-GFP MP = 120.8 ng/uL

Reaction volume	25 uL (x3)	20 uL (x3)	10 uL (x3)	5 uL (x3)	2.5 uL (x3)	1 uL (x3)
Solution A	10	8	4 µL	2 µL	1	0.4
Solution B	7.5	6	3 µL	1.5 µL	0.75	0.3
RNAse inhibitor	0.25	0.2	0.1 µL	0.05	0.025	0.01
H2O	6.345	4.972	2.486	1.243	0.6215	0.25
DNA (5 ng/uL)	1.03	0.828	0.414	0.207	0.1035	0.04

Controls	25 uL (x1)	20 uL (x1)	10 uL (x1)	5 uL (x1)	2.5 uL (x1)	1 uL (x1)
Solution A	10	8	4 µL	2 µL	1	0.4
Solution B	7.5	6	3 µL	1.5 µL	0.75	0.3
RNAse inhibitor	0.25	0.2	0.1 µL	0.05	0.025	0.01

H2O	7.25	5.8	2.9	1.45	0/725	0.29
DNA (5 ng/uL)	0	0	0	0	0	0

T7-eGFP-terminator quick test, 5 uL reactions

Amount of plasmid	200 ng	100 ng	50 ng	0 ng (use above)
Solution A	2 µL	2 µL	2 µL	2 µL
Solution B	1.5 µL	1.5 µL	1.5 µL	1.5 µL
RNAse inhibitor	0.05	0.05	0.05	0.05
water	0.46	0.44	0.83	1.45
DNA	0.99 (eGFP 1)	1.01 (eGFP 3)	0.62 (eGFP 2)	0
rfu's (ex 485 em 515 cutoff 495)	**505.40**	**485.85**	**185.78**	**11.656**

PROCEDURE

1. Master mix: 25 uL x 4
   - 20 uL x 4 = 111.6 uL Solution A
   - 10 uL x 4 = 83.7 uL Solution B
   - 5 uL x 9 = 2.79 uL RNAse inhibitor
   - 2.5 uL x 4
   - 1 uL x 4
2. Remove 3.55 uL x3 for T7-eGFP-term reactions
   - Add appropriate amount of DNA and water
3. From remaining mix, remove 45.085 uL for controls
   - Add 18.415 uL water
   - Separate into 25, 20, 10, 5, 2.5, 1 uL CONTROLS DONE
4. To remaining mix, add 49.86 uL water
   - 8.2815 uL DNA (MP T7-GFP, 120.8 ng/uL)
   - Separate into 3 each of 25, 20, 10, 5, 2.5, 1 uL

Black plate:

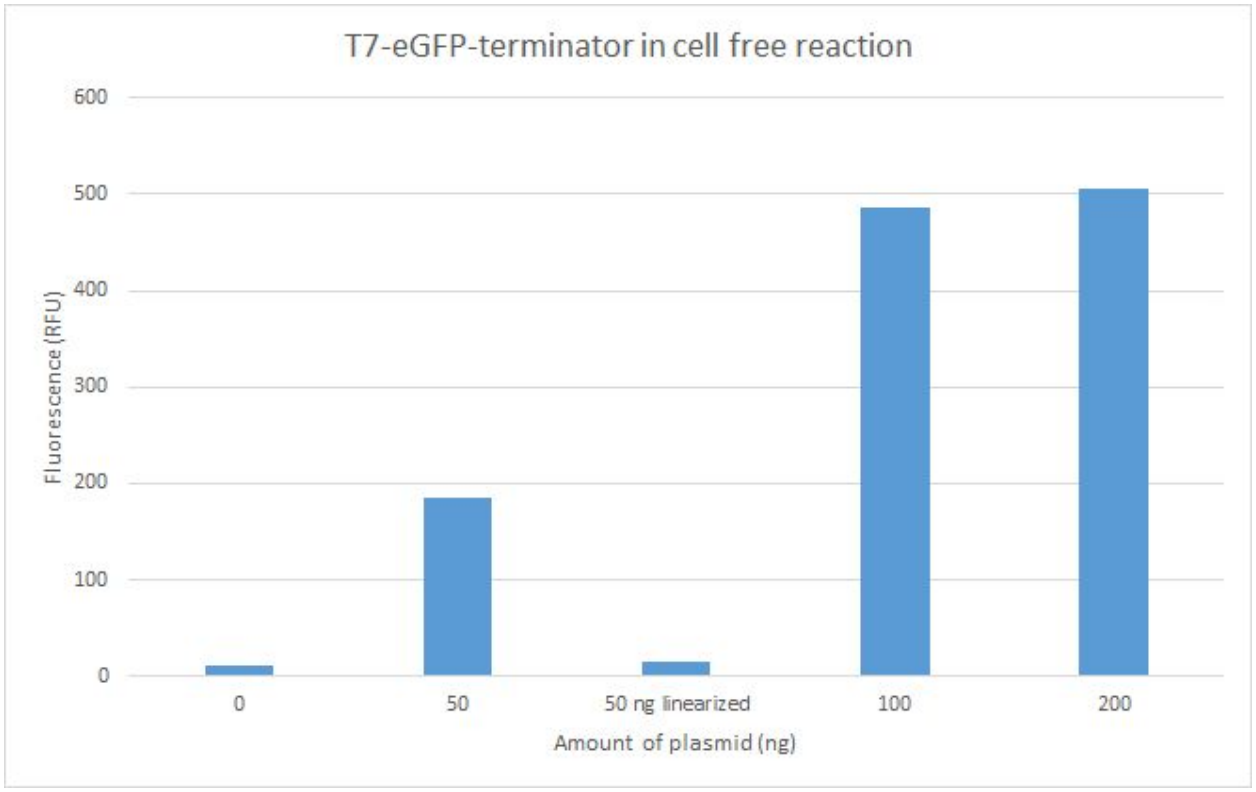
	1	2	3	4	5	6	7	8	9	10	11	12
B					1 C	1-1	1-2	1-3	2.5 C	2.5-1	2.5-2	2.5-3
C	5 C	5-1	5-2	5-3	10 C	10-1	10-2	10-3	20 C	20-1	20-2	20-3
D	25 C	25-1	25-2	25-3	eGFP 50	eGFP 100	eGFP 200					

Plate01

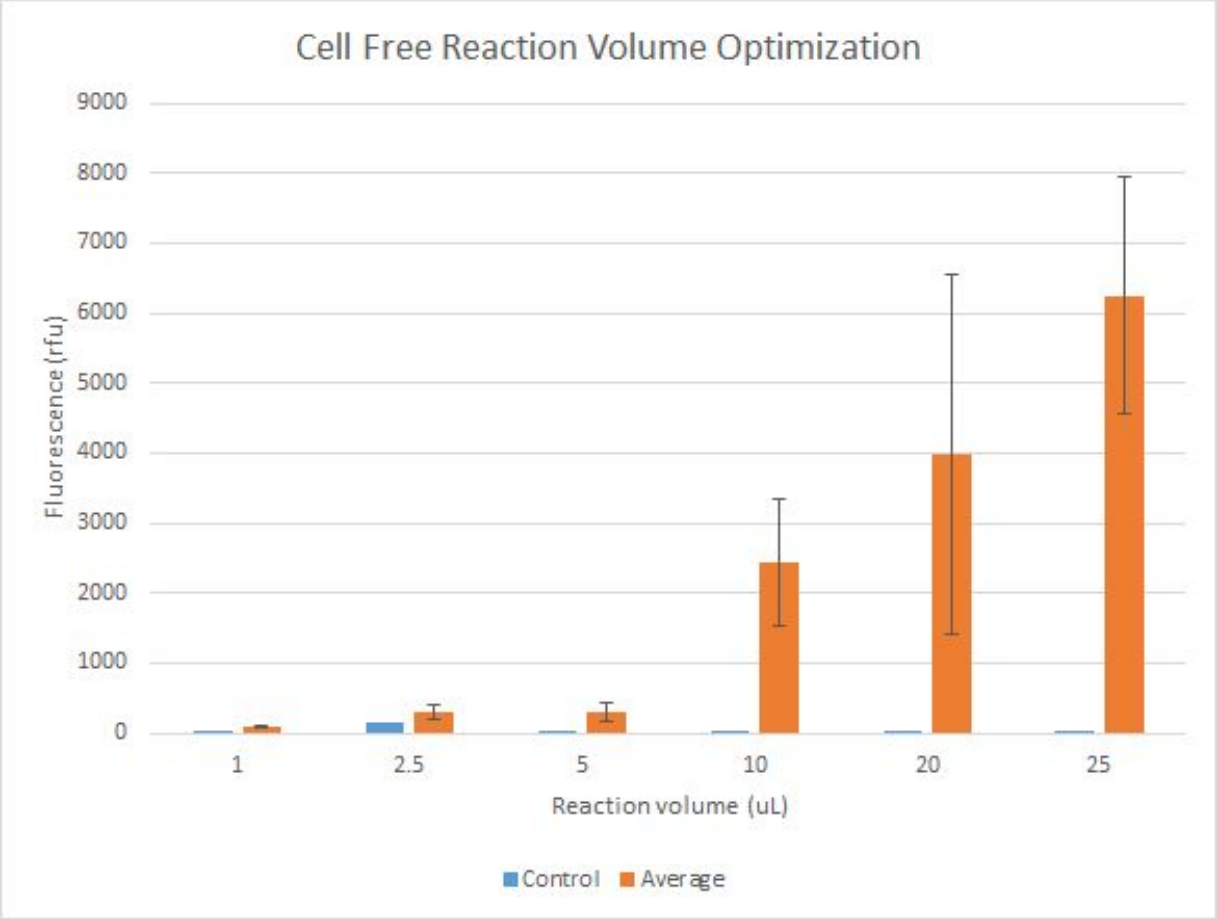
	1	2	3	4	5	6	7	8	9	10	11	12
A	13.924	16.883	12.463	13.010	12.137	392.00	125.45	12.582	85.224	74.561	159.54	112.75
B	57.254	15.707	351.86	351.67	11.763	85.746	124.02	76.468	151.38	244.90	434.46	252.79
C	11.656	437.97	281.60	157.75	17.979	3481.5	2053.2	1785.9	11.929	1745.7	3421.0	6781.2
D	46.705	4560.8	6231.4	7962.7	185.78	485.85	505.40	12.139	14.326	13.321	14.725	15.078

Endpoint
Fluorescence
Ex Em Cutoff
Lm1 485 515 495

RESULTS



Fluorescence read at 485 excitation, 515 emission, 495 cutoff after a 2-hour incubation. 5-uL reactions were diluted to a total of 25 uL to accommodate the plate reader.



Fluorescence read at 485 excitation, 515 emission, 495 cutoff after a 2-hour incubation. Reactions were diluted to a total of 25 uL to accommodate the plate reader.

Cell-free Collins plasmids with DNA trigger quick test (Aife)

Triggers diluted to D: 61.725 ng/uL G: 62.5 ng/uL

	D	G
Solution A	2	2
Solution B	1.5	1.5
RNAse inhibitor	0.25	0.25
Trigger (25 ng)	0.4	0.4
Switch (25 ng)	0.6	0.52
Substrate	0.25	0.25
Water	0	0.8

Absorbance (570 nm)	**0.093**	**0.090**

Controls from day before at 0.064, 0.047
Color darkened, but not fully purple

Cell-free linear T7-eGFP test (Aife)

T7-eGFP digested with PstI and purified

	50 ng linear eGFP	control
Solution A	2	2
Solution B	1.5	1.5
RNAse inhibitor	0.25	0.25
DNA	0.73 at 68.4 ng/uL	0
Water	0.52	1.25
Fluorescence Ex 484 Em 585 Cutoff 495	**13.948**	**31.612**

Linearized without terminator doesn't work, unless control and DNA got mixed up

Cell-free amilCP plasmid quick test again (Aife)

	200 ng plasmid amilCP
Solution A	2
Solution B	1.5
RNAse inhibitor	0.25
DNA	1.34 (amilCP 4)
Water	0
Absorbance (588 nm)	**0.045**, similar to empty wells

No amilCP expression

Plate full-length lacZ

Liquid culture of T7-eGFP-term

Friday, July 1

No lacZ growth on plate

Glycerol stock of T7-eGFP-term (Claire)

Cell-free Collins DNA trigger dosage (Claire)

Trigger oligo dilution (for D and G)

100 uM /4 = 25 uM /10 = **2.5 uM** /10 = **250 nM** /10 = 25 nM /10 = **2.5 nM** /10 = 0.25 nM /10 = 25 pM /10 = **2.5 pM**

D 2.5 uL	8 uM x2	1 uM x2	100 nM x2	1 nM x2	1 pM x2	0	mismatch
Sol'n A	1	1	1	1	1	1	1
Sol'n B	1.5	1.5	1.5	1.5	1.5	1.5	1.5
RNAse in	0.125	0.125	0.125	0.125	0.125	0.125	0.125
trigger	0.4 100 uM	0.4 2.5 uM	0.4 250 nM	0.4 2.5 nM	0.4 2.5 pM	0	0.4 100 uM G
switch	0.6	0.6	0.6	0.6	0.6	0.6	0.6
substrate	0.25	0.25	0.25	0.25	0.25	0.25	0.25
H2O	0	0	0	0	0	0.4	0

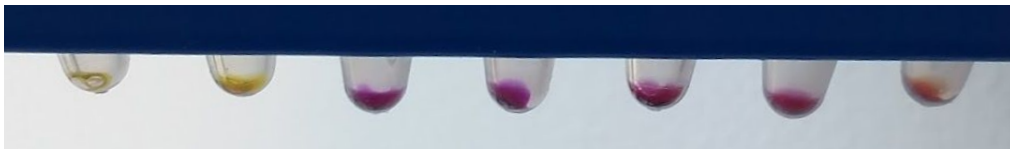
G 2.5 uL	8 uM x2	1 uM x2	100 nM x2	1 nM x2	1 pM x2	0	mismatch
Sol'n A	1	1	1	1	1	1	1
Sol'n B	1.5	1.5	1.5	1.5	1.5	1.5	1.5
RNAse in	0.125	0.125	0.125	0.125	0.125	0.125	0.125
trigger	0.4 100 uM	0.4 2.5 uM	0.4 250 nM	0.4 2.5 nM	0.4 2.5 pM	0	0.4 100 uM D
switch	0.52	0.52	0.52	0.52	0.52	0.52	0.52
substrate	0.25	0.25	0.25	0.25	0.25	0.25	0.25
H2O	0.08	0.08	0.08	0.08	0.08	0.48	0.08

PROCEDURE

1. Master mix: 26 rxns, each 2.5 uL
   - 26 uL Solution A
   - 19.5 uL Solution B
   - 3.25 uL RNAse inhibitor
   - 3.25 uL substrate
2. Separate into 2 x 26 uL
   - D // G
3. Add 3.9 uL // 3.38 uL switch
4. Remove 2 x 2.3 uL // 2 x 2.28 uL for control and mismatch

Control: add 0.2 uL	//	0.24 uL water
Mismatch: add 0.2 uL	//	0.2 uL DNA
		0.04 uL water
5. Separate into 5 x 4.6 uL	//	5 x 4.52 uL
Add 0.4 uL	//	0.4 uL DNA of correct concentration

Incubate for 2 hours, then separate into 2.5 uL reactions diluted to 25 uL in plate
Color change visible for D in all concentrations >= 1 nM by 30 min, 8 uM dark
G 1 pM still quite light 1:30 in, 1 nM visibly darkened



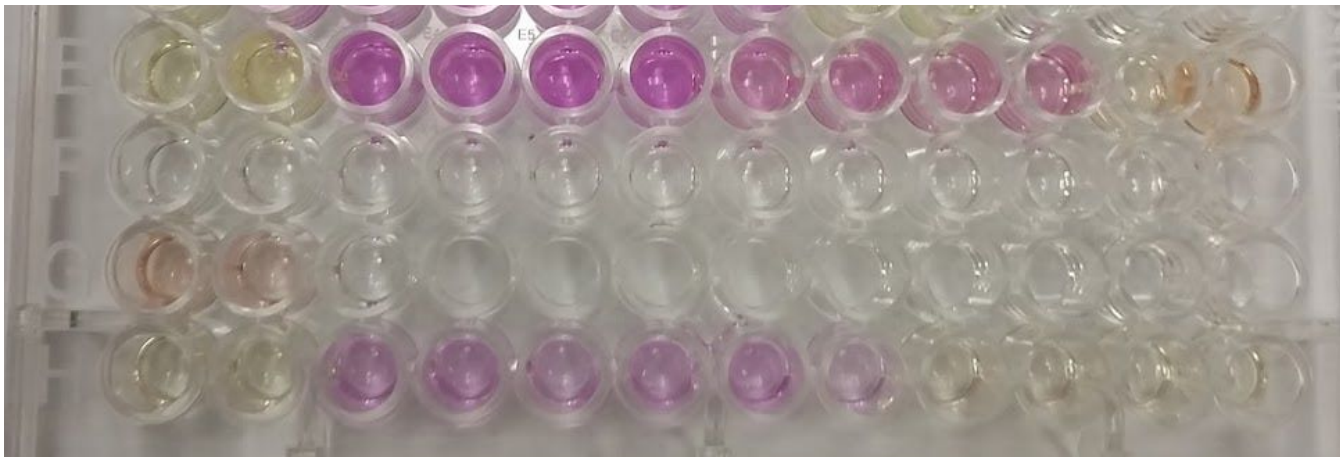
D switch. Control, MM, dec. concentration

G switch. Control, MM, dec. concentration

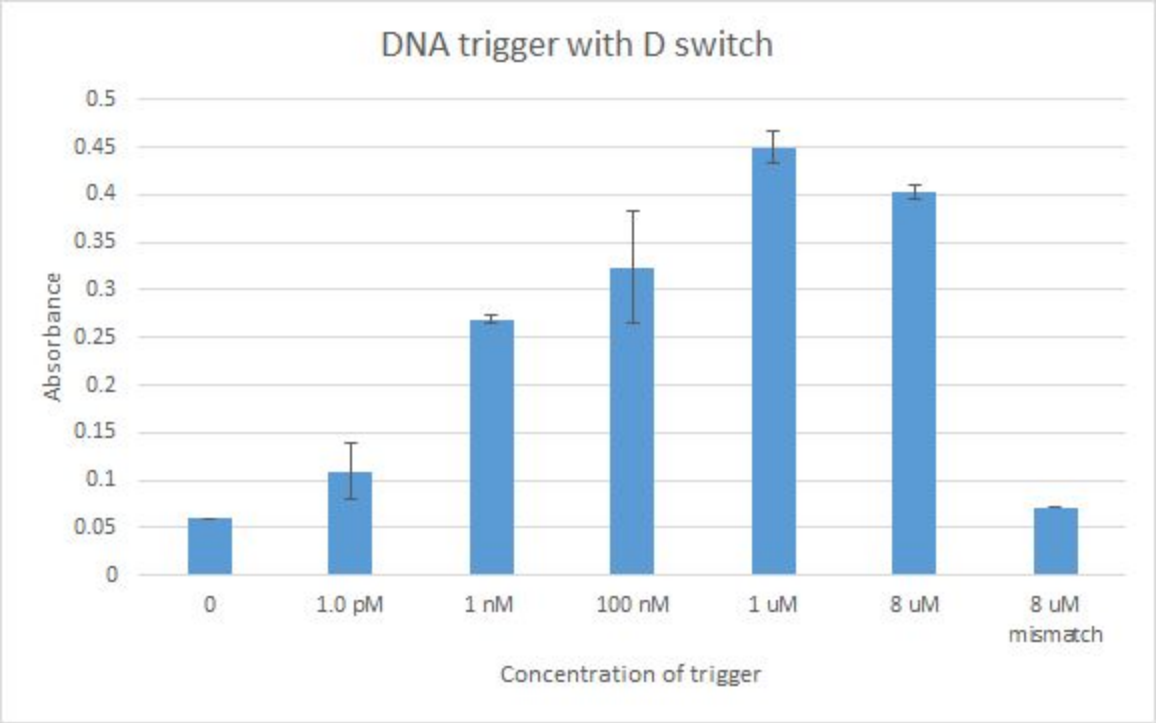
Clear plate

Absorbance 570	Plate	50 uL total, 5 uL rxn (25 total, 2.5 rxn for 0, MM)	25 uL total 2.5 uL rxn	50 uL total 2.5 uL rxn
D 0	E1	0.053	0.056	0.060
D MM	E2	0.068	0.054	0.071
D 8 uM 1	E3	0.808	0.109	0.398
D 8 uM 2	E4	--	0.141	0.408
D 1 uM 1	E5	0.878	0.101	0.462
D 1 uM 2	E6	--	0.117	0.438
D 100 nM 1	E7	0.571	0.075	0.282
D 100 nM 2	E8	--	0.107	0.366
D 1 nM 1	E9	0.514	0.064	0.266
D 1 nM 2	E10	--	0.083	0.272
D 1 pM 1	E11	0.133	0.030	0.088

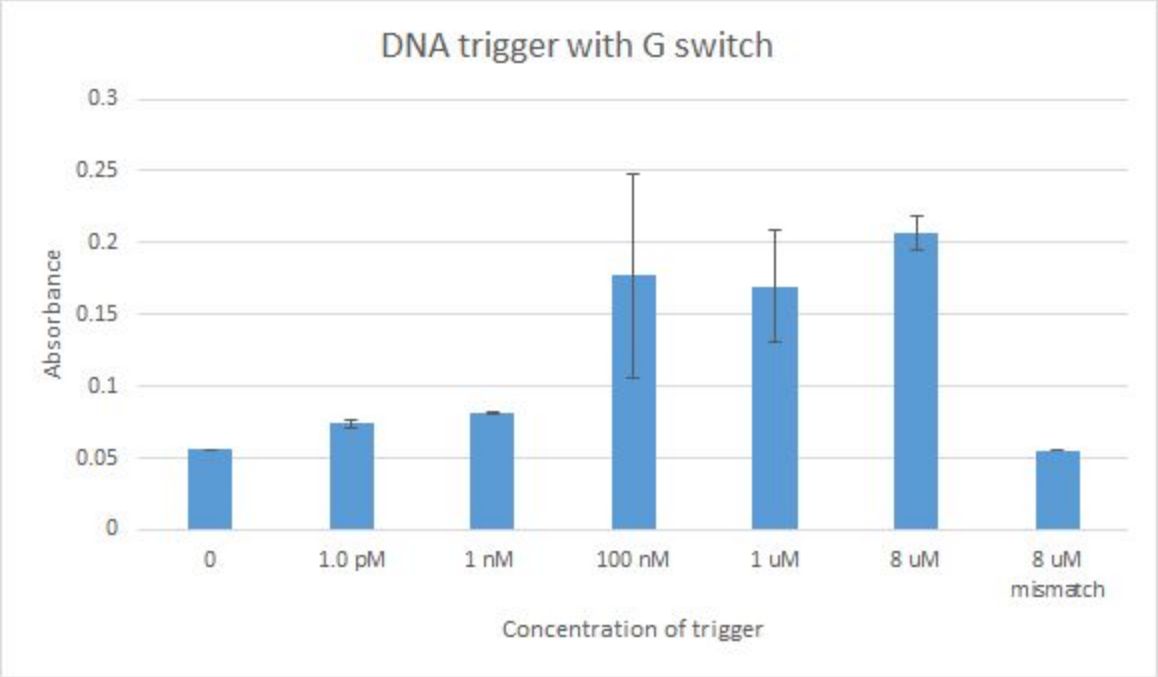
D 1 pM 2	E12	--	0.068	0.130
G 0	H1	0.044	0.042	0.056
G MM	H2	0.047	0.048	0.055
G 8 uM 1	H3	0.383	0.061	0.198
G 8 uM 2	H4	--	0.074	0.215
G 1 uM 1	H5	0.305	0.027	0.142
G 1 uM 2	H6	--	0.067	0.197
G 100 nM 1	H7	0.347	0.094	0.227
G 100 nM 2	H8	--	0.036	0.127
G 1 nM 1	H9	0.102	0.057	0.081
G 1 nM 2	H10	--	0.066	0.082
G 1 pM 1	H11	0.074	0.060	0.076
G 1 pM 2	H12	--	0.071	0.072



RESULTS



Absorbance read at 570 nm for 2.5 uL reactions diluted to 50 uL.



Absorbance read at 570 nm for 2.5 uL reactions diluted to 50 uL.

Cell-free amilCP overnight incubation (Claire)

5 uL rxn	74.6 ng	200 ng	0 ng

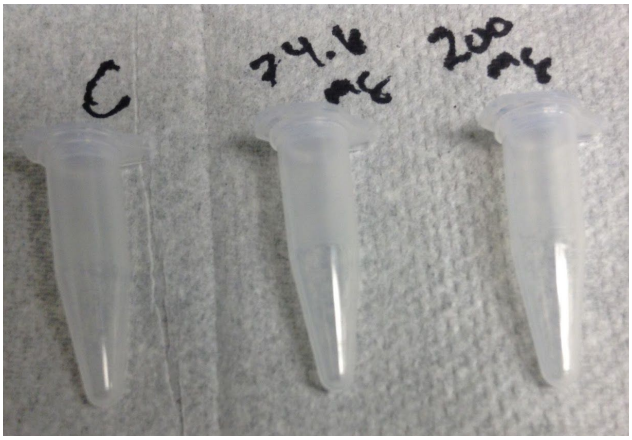
Solution A	2	2	2
Solution B	1.5	1.5	1.5
RNAse	.25	.25	.25
DNA	0.5	1.34	0
water	0.75	0	1.25

After 2 hours, remove 1 uL from each into room temp. Remaining 4 uL in 37

Streak lacZ again

Sunday, July 3

Check amilCP reactions--no color



No growth on lacZ plates